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Morphology of *Leishmania donovani* Colonies Grown on Blood Agar Plates

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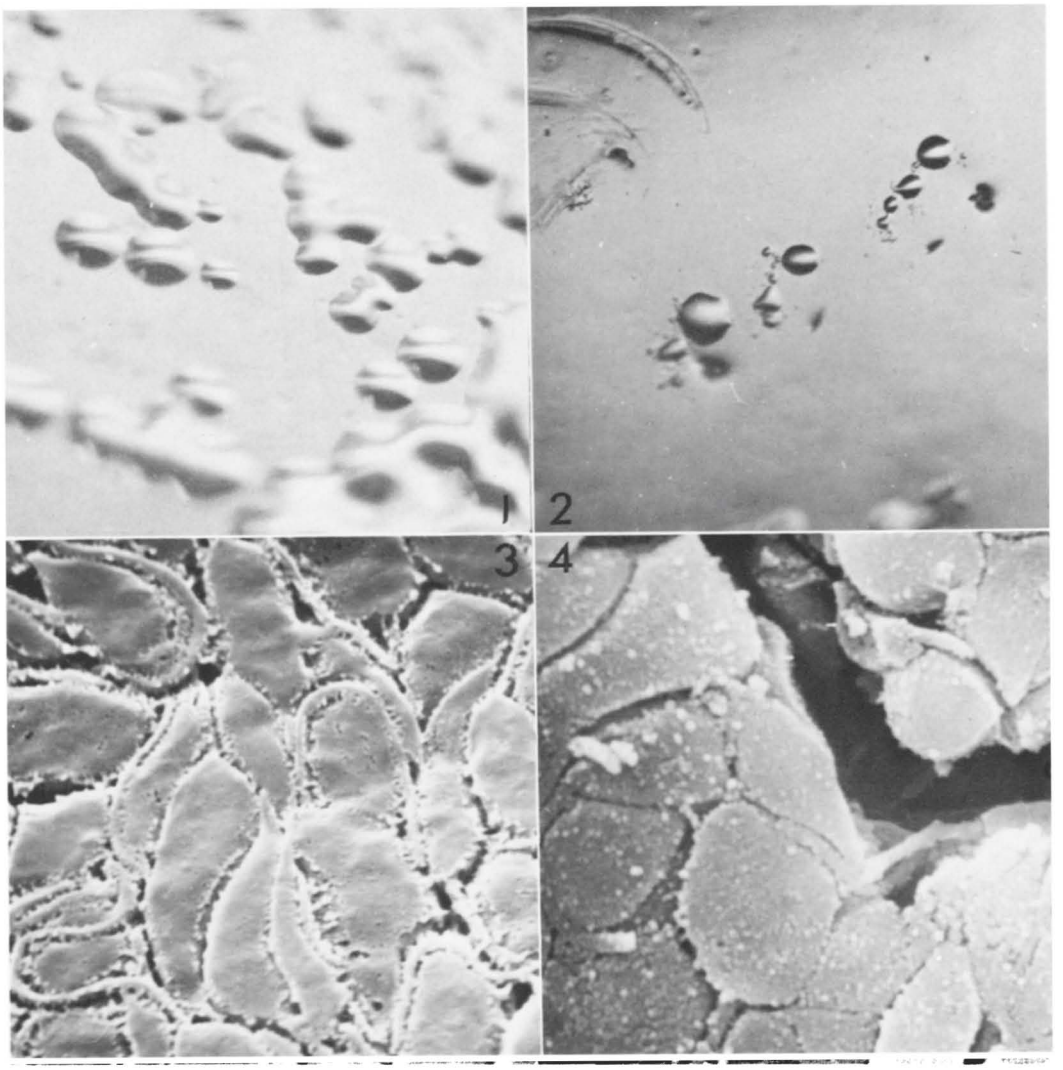
Previous studies have shown that several species of Trypanosomatidae can be grown as discrete and, in some cases clonal, colonies on blood agar plates, and that species differ in colony phenotype (Noeller, 1917, Arch. Schiffs Trop. Hyg. **21:** 5–94; Senekjie, 1944, J. Parasitol. **30:** 303–308; Newton, 1956, Nature **177:** 279–280; Keppel and Janovy, 1977, J. Parasitol. **63:** 879–882). None of these previous studies, however, has addressed the following question: Does the life cycle stage used to initiate agar plate culture affect colony phenotype? The question also can be phrased as follows: Does passage through a vertebrate



FIGURES 1–4. *Leishmania donovani*, 2S strain. **1.** Colonies on blood agar plates at 8 days; promastigote initiated. **2.** Colonies on blood agar plates at 9 days; amastigote initiated from hamster spleen. **3.** SEM of promastigote initiated colony on blood agar plate. ×7,000. **4.** SEM of amastigote initiated colony on blood agar plate. ×7,000.

host alter agar plate colony phenotype? Ultimately, this question must be answered if agar plate colony phenotypes are ever to be used as identification tools in the Trypanosomatidae in a manner similar to that used so successfully for so many years with bacteria and fungi. The present study was intended to address the above question using as parasite material the 2S strain of *Leishmania donovani*.

Laboratory stocks of 2S *L. donovani* were obtained from a primary hamster isolate in 1967 from the late Dr. L. A. Stauber, Rutgers University, and have been maintained since 1967 in Tanabe's medium (Janovy, 1967, Exp. Parasitol. **20:** 51–55) by weekly loop transfer. This stock is referred to as 2S-Stock Culture (2S-SC), and is the same one used in numerous published studies from this laboratory and characterized by Stauber (1966, Ibid. **18:** 1–11). The agar plate culture medium used in all experiments was that described by Keppel and Janovy (1977, loc. cit.). Promastigote-initiated cultures were started using 2S-SC as

previously described (loc. cit.), and amastigote-initiated cultures were begun by touching the cut face of an infected hamster spleen directly on the agar surface. Subsequent incubations were as previously described (loc. cit.). Hamsters used to initiate cultures had received an intracardial injection of 50×10^6 2S-SC promastigotes 20 wk prior to sacrifice.

Colonies were photographed at light level magnifications. They were then fixed for electron microscopy by inverting the entire plate for 24 hr over 2% OsO_4 w/v in distilled water. Fixed colonies were excised on small agar blocks and processed for SEM according to the methods of Current and Janovy (1978, J. Protozool. **25:** 56–65), and examined with a Cambridge Stereoscan S4-10 SEM at 10 kV.

Light- and SEM-level colony phenotypes were described according to a standardized set of criteria used in this laboratory for a variety of trypanosomatid species. These criteria include (1) plating efficiency on medium with human or rabbit blood; (2) time required for colonies to appear to the unaided eye; (3) time required for a full set of phenotypic characters to appear; (4) presence or absence of a colony apron (Keppel and Janovy, 1977, J. Parasitol. **63:** 879–882); (5) colony profile as viewed from the side; (6) colony outline as viewed from the top; (7) colony color and opacity; (8) presence or absence of dark concentric rings within the colony; (9) presence or absence of darkened center; (10) effects on underlying medium; (11) cell packing density; (12) relative amounts of intercellular material; and (13) regularity of cell shape.

Figures 1–4 show light- and SEM-level views of typical colonies of *L. donovani*, 2S strain, initiated from promastigotes and amastigotes. According to the standardized set of criteria used to describe these colonies, no difference could be detected between colonies initiated with promastigotes and those initiated with amastigotes. In both cases, colonies fit the following description. Plating efficiency—60 to 80% on rabbit blood, 0% on human blood; no clouding or bleaching, or other effects on underlying medium; first colonies seen 4 to 6 days post inoculation; full phenotypes develop in 8 to 12 days; single ("clonal") colonies maximum 4 to 5 mm in diameter; color transparent to milky white subopaque; outline subcircular; profile lenticular. Large areas of confluent growth may cover the plate, depending on inoculation technique, with color same as single colonies, but with outline sinuous, profile undulating and irregular. There was no apron on any of the colonies. Cell packing was dense with some imposition of conformation on individuals as a result. There was relatively little intercellular debris. Cells retained some individuality of shape in that both rounded and elongate forms were observed. The outer surface of the colony appeared flattened, possibly as a result of fixation techniques. Flagella were present and lay in grooves between the organisms, not extending above the colony surface. The structural characteristics given above are illustrated in Figures 1–4.

The results indicate that agar plate colonies of *L. donovani*, 2S strain, are indistinguishable regardless of life cycle stage used to initiate the colonies. In addition, this identity extends to the surface architecture as shown by SEM, and includes cell packing arrangement and relative amounts of intercellular material.

Although growth of various trypanosomatids on agar plates has been accomplished many times over the last 50 years, to date no formal attempt has been made to develop a standardized colony description potentially useful in distinguishing species. Use of the SEM reveals a variety of characters not available to earlier workers; thus, the SEM may contribute to development of agar plate colony identification methods not unlike those which have proven so useful to the microbiologists.

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